



Impact of HRC[®] products on aqueous Cr(VI) concentrations and microbial populations in Hanford sediments

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HRC (hydrogen release) compounds release lactate from a polylactate complex at a rate depending on degree of polymerization

HRC primer (pHRC) – rapid lactate release formulation

HRC – original slow release polylactate formulation

HRC-X – extended release polylactate formulation

MRC – metals remediation compound

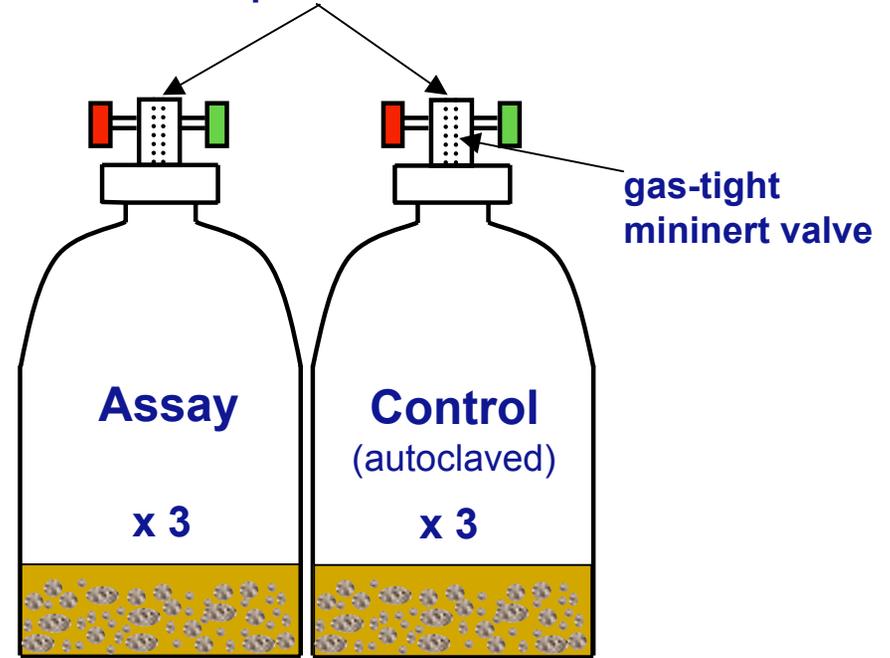
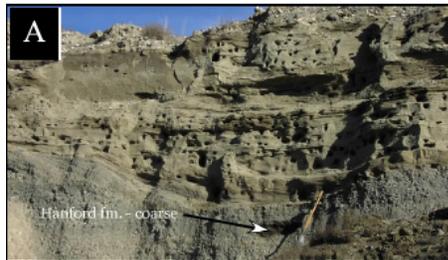
MRC

- glycerol tripolylactate & sorbitol cysteinate
- releases an organosulfur compound upon microbial degradation
- organosulfur compound irreversibly reacts with Cr(VI) producing metal-organosulfur complex which sorbs strongly to sediments

Lactate (Na salt)

Microcosm set up

40 mg C equivalent to 1g HRC kg⁻¹ sediment
No Carbon or Lactate or pHRC or HRC or HRC-X or MRC



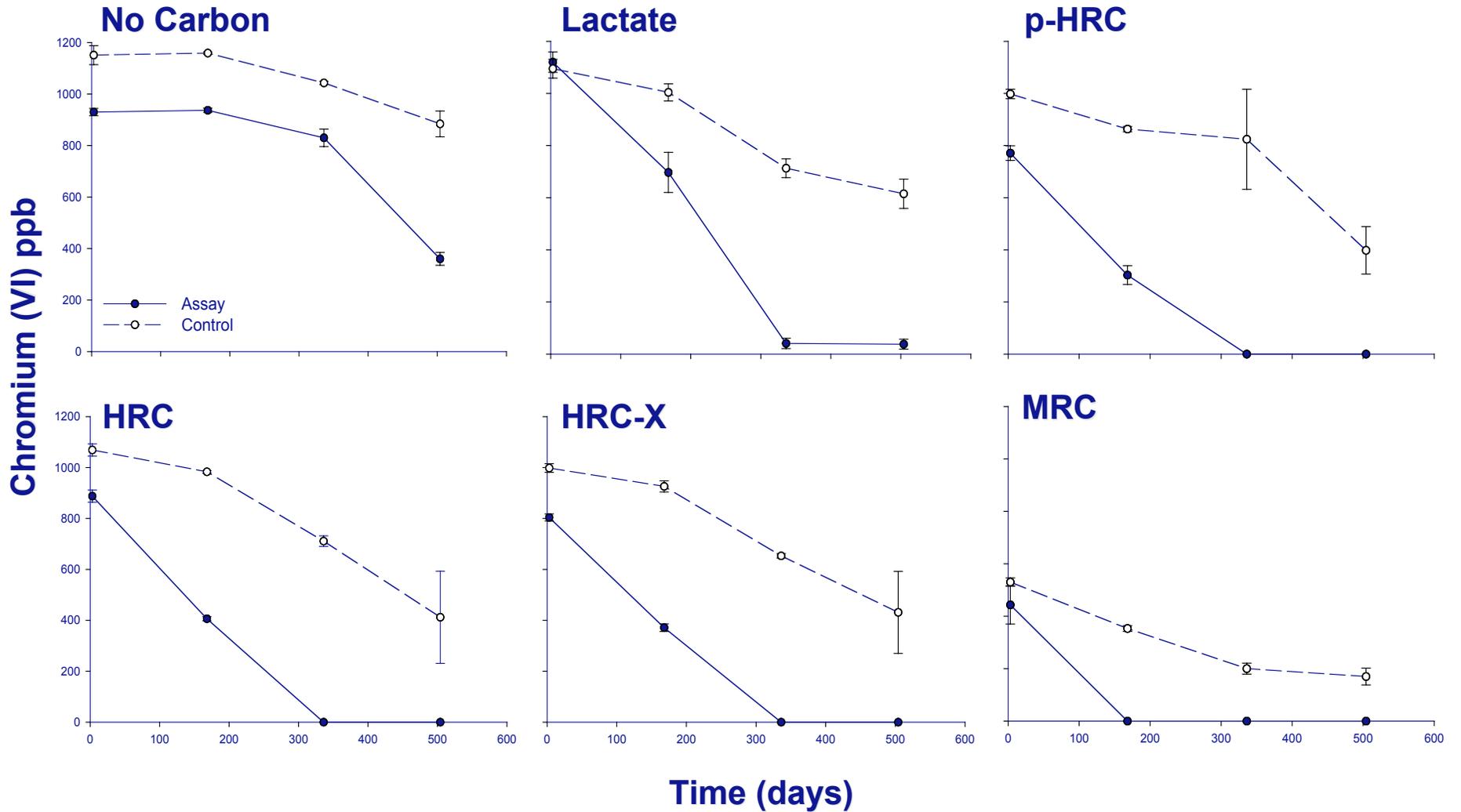
100g Hanford Course Sediment
Groundwater adjusted to 1000 ppb Cr(VI)

Flasks incubated for 3 weeks

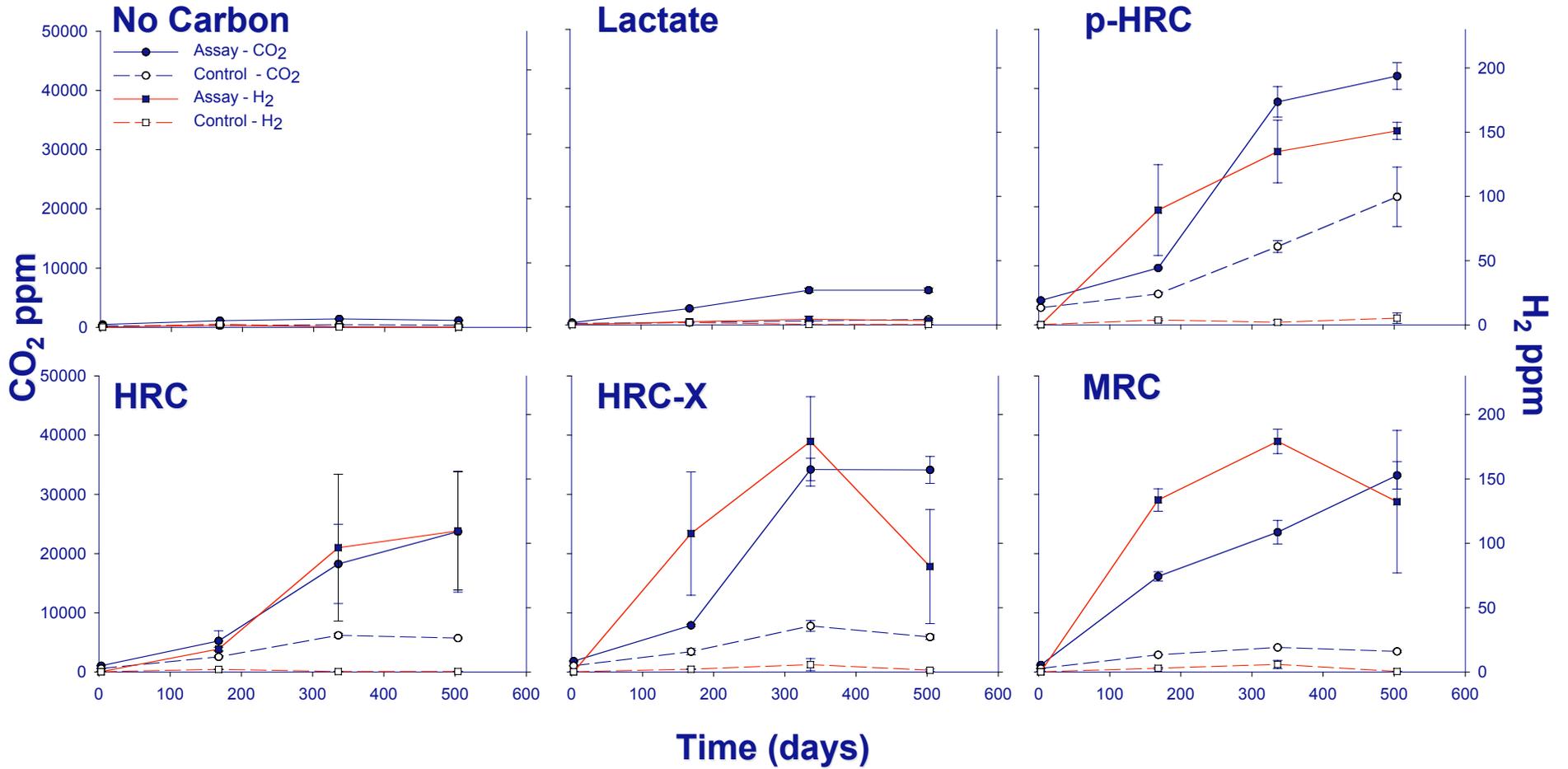
Sampled weekly for: Cr(VI), pH, CO₂, H₂, CH₄, O₂

Sampled after 3 weeks for: Bacterial counts; PLFA and DNA extracted

Effect of HRC products on aqueous Cr(VI) concentration



Effect of HRC products on headspace CO₂ and H₂ concentrations





H₂ headspace concentrations used to determine solution phase H₂ at 3 weeks

No Carbon – 0.12 nM

Lactate – 3 nM

HRC-X – 64 nM

HRC – 85 nM

MRC – 104 nM

p-HRC – 118 nM

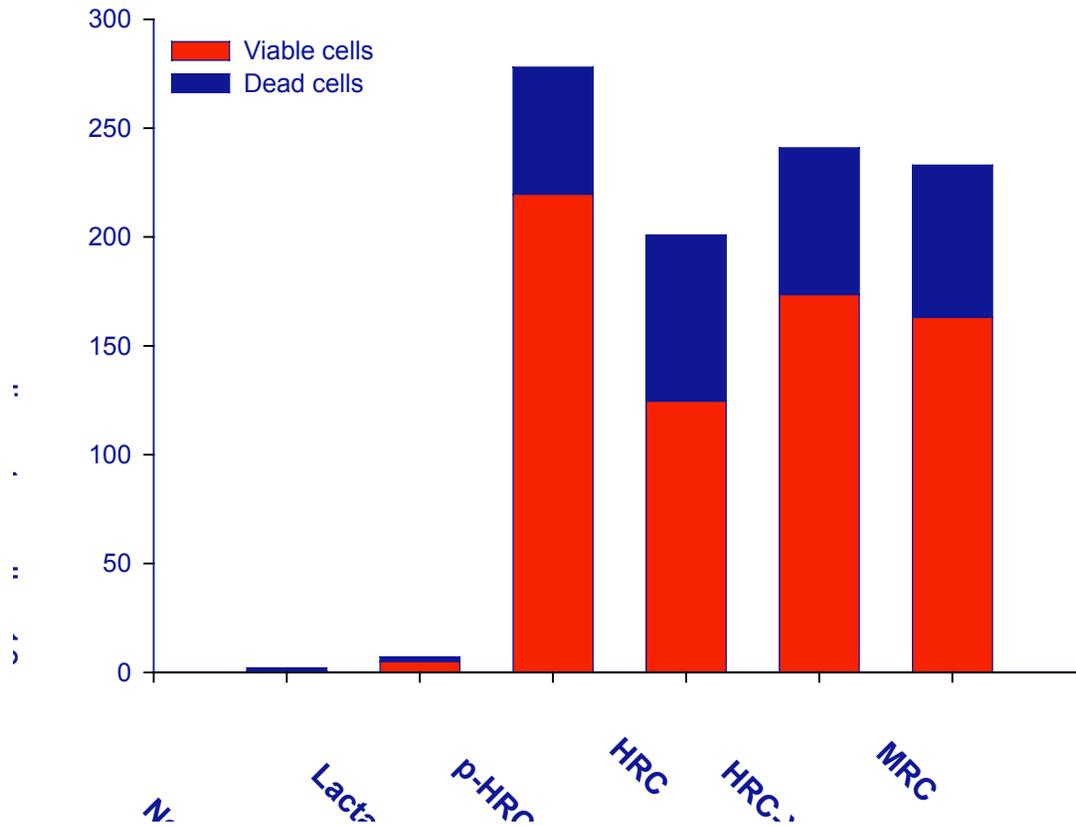
Aqueous H₂ concentrations of 7-10 nM associated with methanogenesis

1-1.5 nM with sulfate reduction

0.2 nM with Fe(III) reduction

<0.05 nM with Mn(IV)/NO₃⁻/Cr(VI) reduction

Effect of HRC products on bacterial numbers and viability



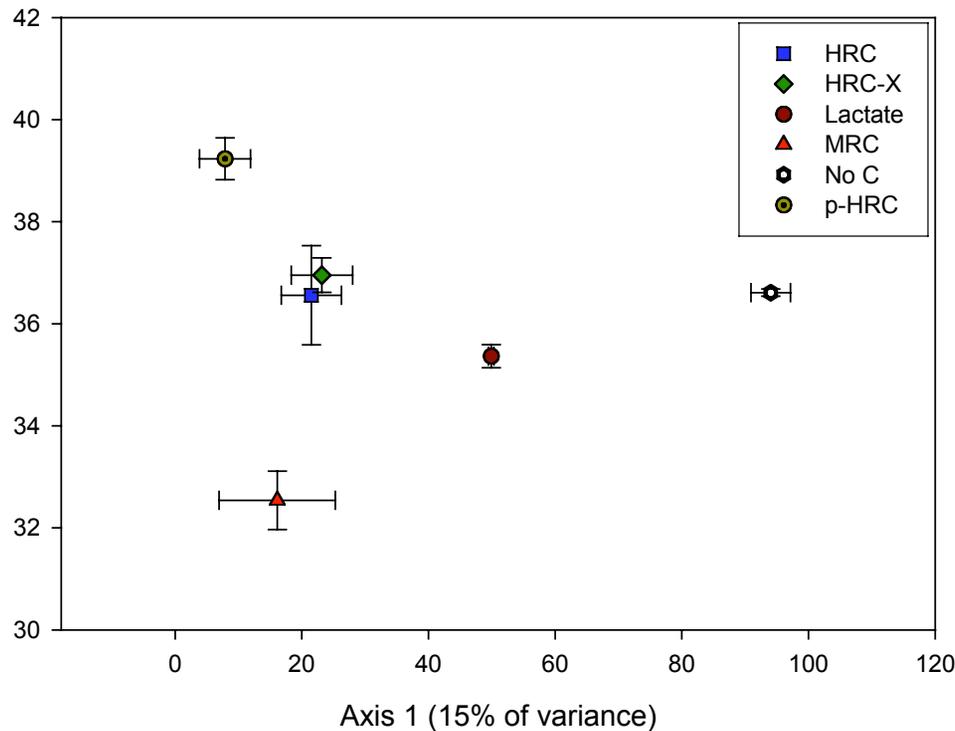
- Bacterial biomass stimulated by added carbon compounds

- HRC products stimulate greater biomass than Na-lactate

- > 100 fold compared with 3 fold

T-RFLP analysis of bacterial community changes with HRC amendment

PCA of community structure following C stimulated Cr(VI) reduction



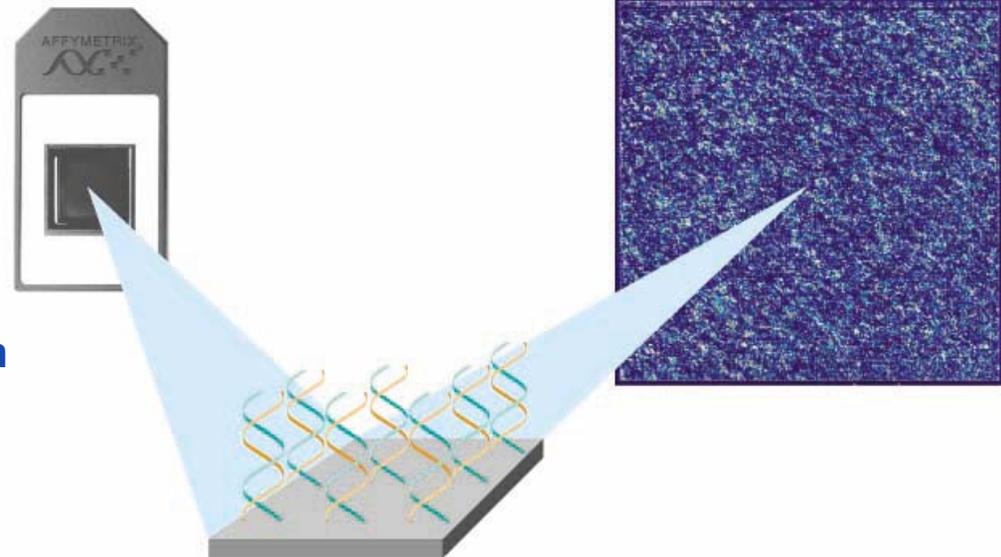
- Bacterial community structure altered by added carbon
- HRC and HRC-X resulted in similar communities
- MRC and p-HRC most different from original community
- Lactate had least effect on bacterial community

High density oligonucleotide microarray analyses

Affymetrix GeneChip

Developed by Gary Andersen at LBNL

- Custom-made 16S rDNA array
- 500,000 probes per array
- Detect 9,121 bacteria/archae
- Monitor changes in composition
- Track species dynamics





High density oligonucleotide microarray analyses

Preliminary data

3 samples analyzed after 3 weeks incubation – No Carbon – HRC – MRC

Dominant organisms confirmed by 16S clone library analysis

No Carbon	HRC	MRC
<i>Arthrobacter</i> sp.	<i>Sporomusa</i> sp.	<i>Sporomusa</i> sp.
<i>Methylophaga</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.
<i>Cellulomonas</i> sp.	<i>Aquaspirillum</i> sp.	<i>Burkholderia</i> sp.
<i>Pseudomonas</i> sp.	<i>Acidovorax</i> sp.	<i>Pseudomonas</i> sp.
<i>Blastomonas</i> sp.	<i>Clostridium</i> sp.	<i>Serratia</i> sp.
<i>Aquaspirillum</i> sp.	<i>Acidobacterium</i> sp.	<i>Clostridium</i> sp.
<i>Acidovorax</i> sp.	<i>Cellulomonas</i> sp.	<i>Nitrospira</i> sp.
<i>Mesorhizobium</i> sp.	<i>Mesorhizobium</i> sp.	<i>Nitrosomonas</i> sp.
<i>Micrococcus</i> sp.	<i>Arthrobacter</i> sp.	<i>DeFluvibacter</i> sp.
<i>Methylophilus</i> sp.	<i>Bacillus</i> sp.	<i>Fulvimonas</i> sp.



Main Findings:

Hanford sediment contains bacterial species:

capable of Cr(VI) reduction

tolerant of high concentrations of heavy metals

capable of chlorinated compound metabolism

All HRC products:

stimulated bacterial biomass and activity

enhanced Cr(VI) removal from solution

resulted in highly reducing conditions

16S rDNA microarrays:

identified diverse bacterial communities after stimulation

permit ~9,000 bacterial species to be monitored during

remediation